

First record of *Theloderma khoii* Ninh, Nguyen, Nguyen, Hoang, Siliyavong, Nguyen, Le, Le & Ziegler, 2022 from China, with confirmation of *Rhacophorus orlovi* Ziegler & Köhler, 2001 in China (Anura, Rhacophoridae)

Shuo Liu^{1,2}, Mian Hou³, Yi Fan⁴, Mingzhong Mo⁵, Dingqi Rao²

¹ Kunming Natural History Museum of Zoology, Chinese Academy of Sciences, Kunming, Yunnan 650223, China

² Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, Yunnan 650201, China

³ College of Continuing (Online) Education, Sichuan Normal University, Chengdu, Sichuan 610066, China

⁴ Yunnan Association for the protection and promotion of natural and cultural heritage, Kunming, Yunnan 650031, China

⁵ Honghe Prefecture Forestry and Grassland Bureau of Yunnan Province, Mengzi, Yunnan 661199, China

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Corresponding author: Dingqi Rao (raodq@mail.kiz.ac.cn)

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Abstract

We report the first country record of *Theloderma khoii* Ninh, Nguyen, Nguyen, Hoang, Siliyavong, Nguyen, Le, Le & Ziegler, 2022 from China based on a specimen collected from Wenshan Prefecture, southeastern Yunnan, China. Morphologically, the specimen from Wenshan Prefecture agrees well with the type specimens of *T. khoii* from Vietnam except for having a smaller body size, and phylogenetically clustered with the type specimens of *T. khoii* from Vietnam. In addition, we confirm the distribution of *Rhacophorus orlovi* Ziegler & Köhler, 2001 in China based on three specimens collected from Honghe Prefecture, southern Yunnan, China. Morphologically, the specimens from Honghe Prefecture agree well with the type specimens of *R. orlovi* from Vietnam except for having a slightly larger body size in adult females, and phylogenetically clustered with the specimens of *R. orlovi* from Vietnam (including the type locality of *R. orlovi*).

Key Words

16S rRNA, Honghe Prefecture, Wenshan Prefecture, Yunnan

Introduction

The genus *Theloderma* Tschudi is a poorly known group of tree frogs which presently comprising 28 species distributed throughout Southeast Asia, southern China and northeastern India (Hou et al. 2017; Poyarkov et al. 2018; Du et al. 2022; Frost 2022; Ninh et al. 2022). To date, ten species have been reported from China (Du et al. 2022), namely *T. albopunctatum* (Liu & Hu), *T. baibungense* (Jiang, Fei & Huang), *T. bicolor* (Bourret), *T. corticale*

(Boulenger), *T. gordonii* Taylor, *T. hekouense* Du, Wang, Liu & Yu, *T. lateriticum* Bain, Nguyen & Doan, *T. moloch* (Annandale), *T. pyaukkya* Dever, and *T. rhododiscus* (Liu & Hu). *Theloderma khoii* Ninh, Nguyen, Nguyen, Hoang, Siliyavong, Nguyen, Le, Le & Ziegler, 2022 is a species which was described recently from Ha Giang Province, Vietnam, and is currently known only from its type locality.

The genus *Rhacophorus* Kuhl & Van Hasselt currently containing 44 species with a distribution range from

India, Bangladesh, Bhutan, Myanmar, Thailand, Laos, Cambodia, Vietnam, Malaysia, Indonesia and Philippines, as well as extreme southern and south-western China (Jiang et al. 2019; Frost 2022; Kropachev et al. 2022). *Rhacophorus orlovi* Ziegler & Köhler, 2001 is a species which was described from Ha Tinh Province, Vietnam, and was previously recorded in China by Li et al. (2013) based on only molecular data of two specimens from Maguan County, Wenshan Prefecture, Yunnan Province, without morphological description and detailed collection information. Subsequently, Gao and Sun (2016, 2017) put this species in the checklist of biological species of Yunnan Province without mentioning any information of specific localities or voucher specimens. Poyarkov et al. (2021) also recorded *R. orlovi* from China without reference to any voucher specimens or specific localities. Frost (2022) considered that *R. orlovi* is possibly distributed in China.

During our field surveys in Yunnan, China, a specimen of *Theloderma* was collected from Xichou County, Wenshan Prefecture, southeastern Yunnan, in 2012, and three specimens of *Rhacophorus* were collected from Hekou County, Honghe Prefecture, southern Yunnan, in 2021 (Fig. 1). Detailed morphological comparisons and molecular analysis indicated the specimen from Xichou County to be *T. khoii*, and the specimens from Hekou County to be *R. orlovi*. Herein, we report the new record of *T. khoii* for China and confirm *R. orlovi* in China.

Materials and methods

Specimens were collected, euthanized, and then fixed in 75% ethanol for storage after taking photographs. Liver tissue samples were preserved in 99% ethanol for molecular analysis. All specimens were deposited at Kunming Institute of Zoology, Chinese Academy of Sciences (KIZ).

Genomic DNA was extracted from liver tissue preserved in 99% ethanol using the standard phenol-chloroform extraction protocol (Sambrook et al. 1989). Partial fragment of the mitochondrial 16S rRNA was amplified via the polymerase chain reaction (PCR) using the primers 16SAR: 5'-CGCCTGTTTAYCAAAAACAT-3' and 16SBR: 5'-CCGGTYTGAACATCAGATCAYGT-3' (Palumbi et al. 1991). The product was purified and sequenced by Tsingke Biotechnology (Beijing) Co., Ltd. The new sequences were deposited in GenBank. Species of the genera *Nyctixalus* Boulenger and *Zhangixalus* Li, Jiang, Ren & Jiang were selected as outgroups respectively according to Li et al. (2019) and Du et al. (2022). Homologous and outgroup sequences were obtained from GenBank (Table 1). Sequences were aligned using MAFFT 7 (Katoh and Standley 2013). The technical computation methods for the best substitution model selection, Bayesian inference and Maximum likelihood phylogenetic analyses were the same as those in Liu et al. (2021).

Measurements were taken with a digital caliper to the nearest 0.1 mm. Morphological terminology for *Theloderma khoii* followed Ninh et al. (2022): SVL, snout-vent length; HW, maximum head width (across angle of jaws); HL, head length (from the back of the mandible to the tip of the snout); SNL, snout length (from anterior corner of eye to the tip of the snout); ED, eye diameter; UEW, maximum width of upper eyelid; IN, internarial distance; IOD, interorbital distance; DAE, distance between anterior corners of eyes; DPE, distance between posterior corners of eyes; EN, distance from anterior corner of the eye to the nostril; TYD, tympanum diameter; TYE, distance from anterior margin of tympanum to posterior corner of the eye; FLL, forelimb length (from axilla to elbow); HAL, hand length (from elbow to the tip of third finger); OPT, outer palmar tubercle length; TFL, third finger length; FeL, femur length (from vent to knee); TbL, tibia length (from knee to tarsus); FoL, foot length (from inner metatarsal tubercle to the tip of fourth toe); FTL, fourth toe length; and IMT, inner metatarsal tubercle length. Morphological terminology for *Rhacophorus orlovi* followed Ziegler and Köhler (2001): KRL, snout-vent length; KB, head width; KL, head length; AN, distance between anterior angle of eye to center of nostril; NS, distance between center of nostril to tip of snout; AD, horizontal eye diameter; TD, horizontal tympanum diameter; HaL, length of hand, up to the tip of the longest (3rd) finger; BL, length of the extended hindlimb from cloaca to tip of longest toe; FL, length of foot, exclusive of tarsus, up to the tip of the longest (fourth) toe; and IMT, length of inner metatarsal tubercle.

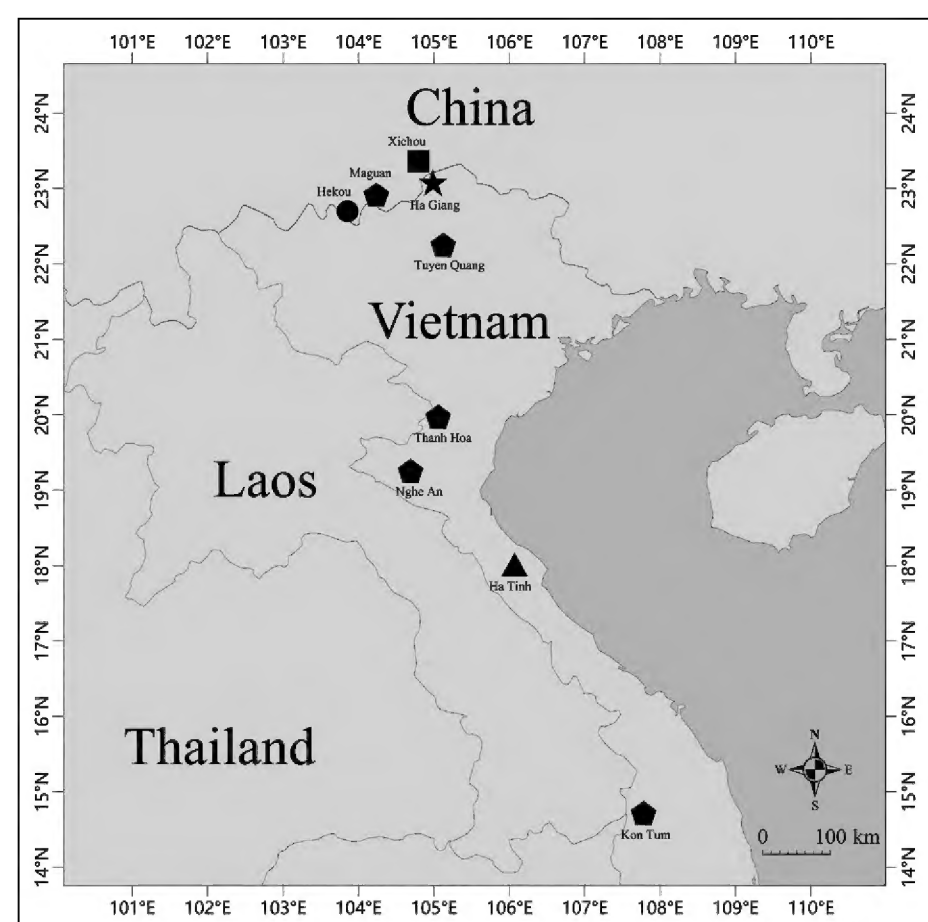


Figure 1. Map showing the type locality (black star) of *Theloderma khoii* and the type locality (black triangle) of *Rhacophorus orlovi* in Vietnam, the new collection site (black square) of *T. khoii* and the new collection site (black dot) of *R. orlovi* in China, and the other localities (black pentagon) of *R. orlovi* whose sequences were used in the phylogenetic analysis.

Table 1. Samples used in molecular analyses of this study.

Taxon	Voucher No.	Locality	GenBank No.
<i>Rhacophorus annamensis</i>	VNMN 4090	Dak Nong, Nam Nung, Vietnam	LC010566
<i>Rhacophorus baluensis</i>	FM235958	Sabah, Malaysia	KC961089
<i>Rhacophorus bengkuluensis</i>	UTA A-62770	Lampung, Sumatra, Indonesia	KM212948
<i>Rhacophorus bipunctatus</i>	CAS229913	Putao, Kachin, Myanmar	JX219445
<i>Rhacophorus borneensis</i>	BORN:22410	Maliau Basin, Sabah, Malaysia	AB781693
<i>Rhacophorus calcaneus</i>	VNMN 4093	Dak Lac, Chu Yang Sin, Vietnam	LC010573
<i>Rhacophorus catamitus</i>	ENS 14726	Sumatra, Indonesia	KX398877
<i>Rhacophorus exechopygus</i>	VNMN 4107	Gia Lai, Kon Ka Kinh, Vietnam	LC010585
<i>Rhacophorus helenae</i>	AMS R 173230	Binh Thuan, Vietnam	JQ288087
<i>Rhacophorus hoabinhensis</i>	VNMN A.2016.16	Hoa Binh, Vietnam	LC331097
<i>Rhacophorus indonesiensis</i>	MZB:Amp:23619	Indonesia	AB983367
<i>Rhacophorus kio</i>	VNMN 4110	Gia Lai, Kon Ka Kinh, Vietnam	LC010589
<i>Rhacophorus lateralis</i>	SDB.2010.330	Karnataka, Bygoor, India	KC571277
<i>Rhacophorus malabaricus</i>	Rmal-In	Madikeri, India	AB530549
<i>Rhacophorus margaritifer</i>	ENS 16162	Java, Indonesia	KX398889
<i>Rhacophorus modestus</i>	ENS 16853	Sumatra, Indonesia	KX398904
<i>Rhacophorus nigropalmatus</i>	Rao081203	Malaysia	JX219438
<i>Rhacophrus norhayatiae</i>	NNRn	Endau Rompin, Johor, Malaysia	AB728191
<i>Rhacophorus orlovi</i>	VNMN 4114	Xuan Lien, Thanh Hoa, Vietnam	LC010597
<i>Rhacophorus orlovi</i>	VNMN 3067	Huong Son, Ha Tinh, Vietnam	LC010598
<i>Rhacophorus orlovi</i>	VNMN 4116	Ngoc Linh, Kon Tum, Vietnam	LC010599
<i>Rhacophorus orlovi</i>	VNMN 4115	Pu Huong, Nghe An, Vietnam	LC010600
<i>Rhacophorus orlovi</i>	VNMN 24628	Ha Tinh, Vietnam	LC545588
<i>Rhacophorus orlovi</i>	VNMN 24626	Ha Tinh, Vietnam	LC545589
<i>Rhacophorus orlovi</i>	TQ.2018.20	Tuyen Quang, Vietnam	LC548743
<i>Rhacophorus orlovi</i>	TQ.2018.56	Tuyen Quang, Vietnam	LC548744
<i>Rhacophorus orlovi</i>	AMNH A161405	Huong Son, Ha Tinh, Vietnam	DQ283049
<i>Rhacophorus orlovi</i>	RAO 03309	Maguan, Yunnan, China	JX219435
<i>Rhacophorus orlovi</i>	LJT R44	Maguan, Yunnan, China	KC465840
<i>Rhacophorus orlovi</i>	KIZ 20210506	Hekou, Yunnan, China	OP393173
<i>Rhacophorus orlovi</i>	KIZ 20210507	Hekou, Yunnan, China	OP393174
<i>Rhacophorus orlovi</i>	KIZ 20210508	Hekou, Yunnan, China	OP393175
<i>Rhacophorus pardalis</i>	FMNH273243	Sarawak, Bintulu, Malaysia	JX219454
<i>Rhacophorus poecilonotus</i>	ENS 16480	Sumatra, Indonesia	KX398920
<i>Rhacophorus pseudomalabaricus</i>	SDB.2011.1010	Kerala, Kadalar, India	KC593855
<i>Rhacophorus reinwardtii</i>	Rao081205	Malaysia	JX219443
<i>Rhacophorus rhodopus</i>	SCUM 060692L	Mengyang, Yunnan, China	EU215531
<i>Rhacophorus robertingeri</i>	VNMN 4123	Gia Lai, Kon Ka Kinh, Vietnam	LC010613
<i>Rhacophorus spelaeus</i>	IEBR A.2011.1	Khammouan, Lao	LC331095
<i>Rhacophorus translineatus</i>	Rao6237	Medog, Tibet, China	JX219449
<i>Rhacophorus vampyrus</i>	VNMN 4125	Hon Ba, Khanh Hoa, Vietnam	LC010616
<i>Rhacophorus verrucopus</i>	6254 Rao	Medog, Tibet, China	JX219436
<i>Theloderma albopunctatum</i>	VNMN JR2887	Vinh Phuc, Vietnam	KU244375
<i>Theloderma annae</i>	NAP05558	Hoa Binh, Vientam	MG917766
<i>Theloderma asperum</i>	ZRC1.1.9321	Malaysia	GQ204725
<i>Theloderma auratum</i>	ZMMU A5828	Gia Lai, Vietnam	MG917767
<i>Theloderma baibungense</i>	YPX31940	Motuo, Tibet, China	KU981089
<i>Theloderma bicolor</i>	VNMN 3536	Lao Cai, Vietnam	KJ802915
<i>Theloderma corticale</i>	MVZ 223905	Vinh Phuc, Vietnam	KU244364
<i>Theloderma gordonii</i>	MVZ 226469	Vinh Phuc, Vietnam	KU244363
<i>Theloderma hekouense</i>	GXNU YU000496	Hekou, Yunnan, China	OL843967
<i>Theloderma horridum</i>	KUHE 52582	Negeri Sembilan, Malaysia	LC012861
<i>Theloderma khoii</i>	VNMN 012757	Ha Giang, Viet Nam	LC641701
<i>Theloderma khoii</i>	VNMN 012758	Ha Giang, Viet Nam	LC641702
<i>Theloderma khoii</i>	KIZRao201204	Xichou, Yunnan, China	OP393172
<i>Theloderma lacustrinum</i>	NCSM 84683	Vientiane, Laos	KX095246
<i>Theloderma laeve</i>	NAP01644	Lam Dong, Vietnam	KT461907
<i>Theloderma lateriticum</i>	VNMN 1216	Bac Giang, Vietnam	LC012851
<i>Theloderma leporosum</i>	LJT W46	Malaysia	KC465841
<i>Theloderma licin</i>	MVZ 9458	Indonesia	KU244368
<i>Theloderma moloch</i>	GXNU YU000115	Yingjiang, Yunnan, China	MT509809
<i>Theloderma nebulosum</i>	ROM 39588	Kon Tum, Vietnam	KT461887
<i>Theloderma palliatum</i>	NAP02516	Lam Dong, Vietnam	KT461903
<i>Theloderma petilum</i>	HNUE MNA2012.0001	Dien Bien, Vietnam	KJ802925
<i>Theloderma phrynoderma</i>	CAS247910	Myanmar	KJ128283
<i>Theloderma pyaukkya</i>	GXNU YU000116	Yingjiang, Yunnan, China	MT509810
<i>Theloderma rhododiscus</i>	CIB GX200807017	Jinxiu, Guangxi, China	LC012842
<i>Theloderma ryabovi</i>	VNMN 3924	Kon Tum, Vietnam	LC012860
<i>Theloderma stellatum</i>	Stell	Chanthaburi, Thailand	KT461918
<i>Theloderma truongsongense</i>	VNMN 4402	Khanh Hoa, Vietnam	LC012847
<i>Theloderma vietnamense</i>	AMS R174047	Mondol Kiri, Cambodia	JN688171
<i>Nyctixalus pictus</i>	KUHE 53517	Malaysia	LC012863
<i>Nyctixalus spinosus</i>	ACD 1043	Mindanao, Philippine	DQ283114
<i>Zhangixalus dennysi</i>	SCUM 060401L	Shaoguan, Guangdong, China	EU215545
<i>Zhangixalus dugritei</i>	SCUM 051001L	Baoxing, Sichuan, China	EU215541

Results

Inferred from partial 16S rRNA fragments, the sequence of the specimen collected from Xichou County, Yunnan, China, clustered with the sequences of the type specimens of *Theloderma khoii* from Vietnam (Fig. 2). There was no genetic distance (uncorrected p-distance) between the sequence of the specimen from Xichou County and the sequences of the type specimens of *T. khoii* from Vietnam. The sequences of the specimens collected from Hekou County, Yunnan, China, clustered with the sequences of the specimens of *R. orlovi* from Vietnam (including the type locality of *R. orlovi*) and from Maguan County, Yunnan, China (Fig. 3). The genetic distance (uncorrected p-distance) between the sequences of the specimens from Hekou County and the sequences of the specimens of *R. orlovi* from Vietnam was 0.4%, the genetic distance (uncorrected p-distance) between the sequences of the specimens from Maguan County and the sequences of the specimens of *R. orlovi* from Vietnam was 0.5%, and there was no genetic distance (uncorrected p-distance) between the sequences of the specimens from Hekou County and the sequences of the specimens from Maguan County.

Morphologically, the specimen from Xichou County agrees well with the type specimens of *T. khoii* from

Vietnam except for having a smaller body size and the specimens from Hekou County agree well with the type specimens of *R. orlovi* from Vietnam except for having a slightly larger body size in adult females.

Taxonomic account

Theloderma khoii Ninh, Nguyen, Nguyen, Hoang, Siliyavong, Nguyen, Le, Le & Ziegler, 2022

Figs 4, 5

Specimen examined. KIZRao201204, subadult female, collected in April 2012 by Yi Fan from Fadou Township, Xichou County, Wenshan Prefecture, Yunnan Province, China (ca. 23°22'N, 104°46'E, 1600 m elevation).

Description of the specimen from China. Measurements are presented in Table 2. Body size small (SVL 29.4 mm); head strongly depressed, length and width almost equal (HL 12.4 mm, HW 12.7 mm); snout short (SNL 4.9 mm), pointed and truncated; eye large (ED 3.8 mm), pupil circular; canthus rostralis indistinct, loreal region oblique, slightly concave; interorbital distance wider than internarial distance and upper eyelid width (IOD 3.4 mm,

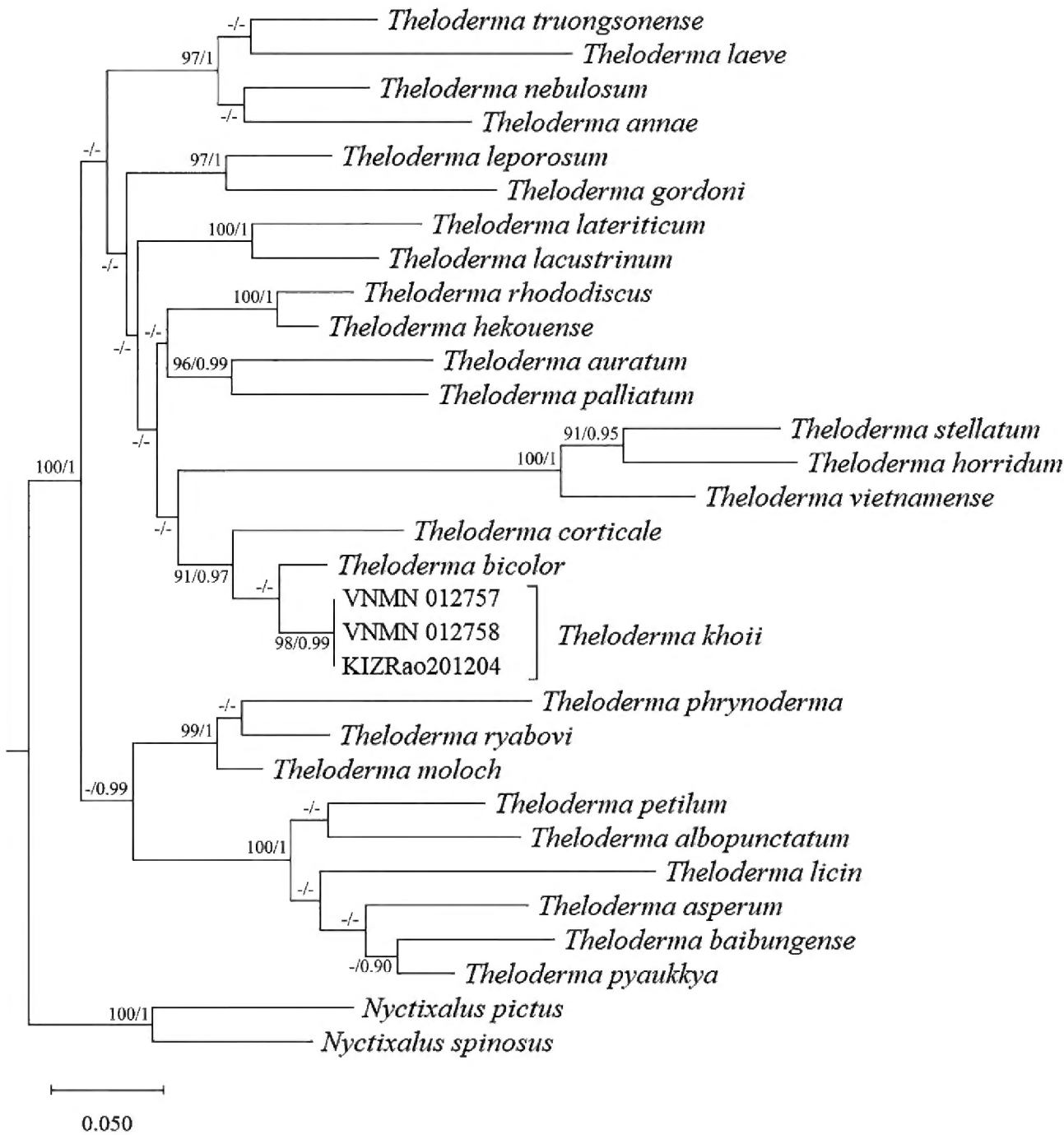


Figure 2. Maximum likelihood tree of the genus *Theloderma* based on partial 16S rRNA fragments. Numbers before slashes indicate ultrafast bootstrap support for Maximum Likelihood analyses (≥ 90 remain) and numbers after slashes indicate Bayesian posterior probabilities (≥ 0.90 remain).

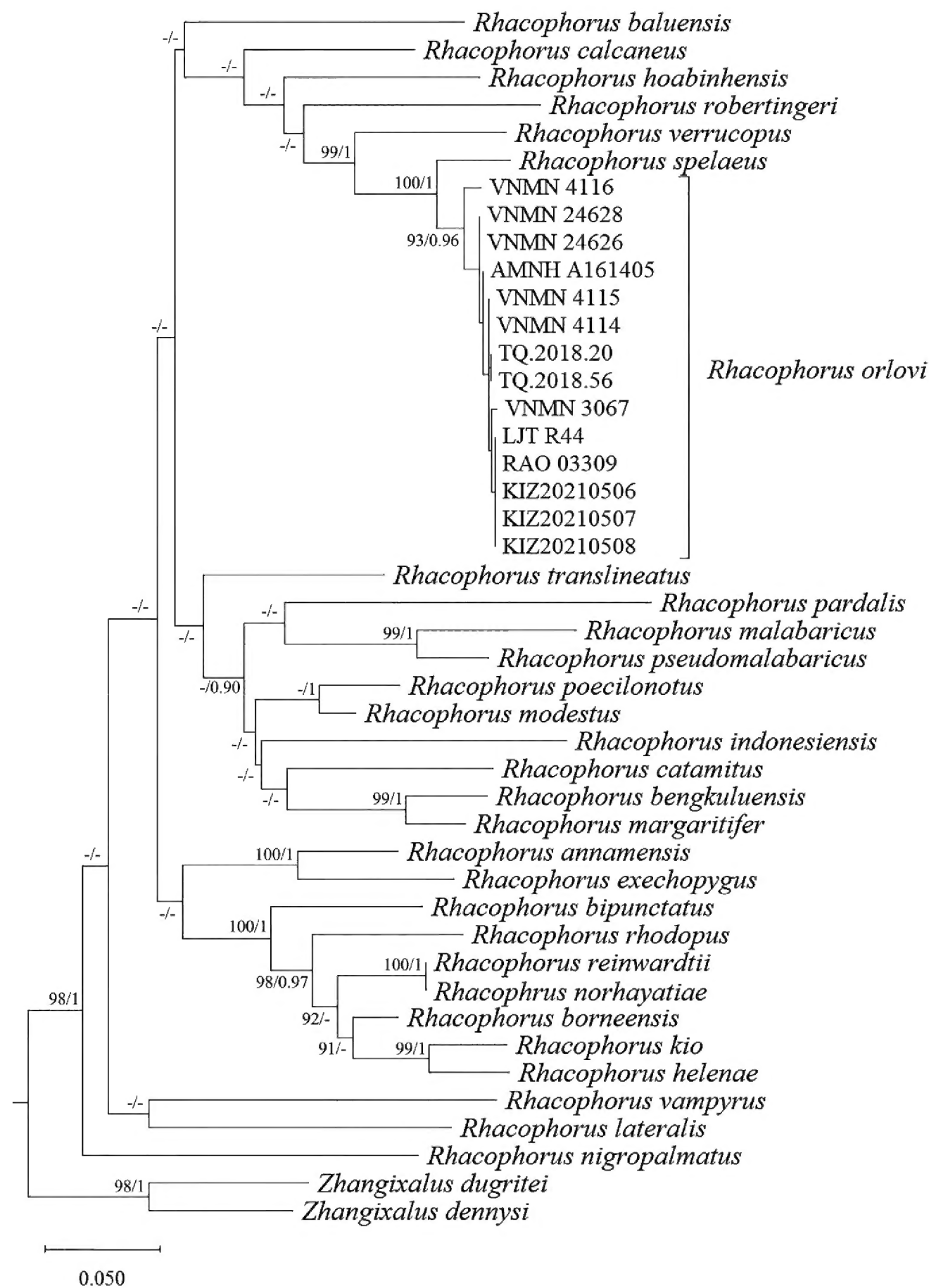


Figure 3. Maximum likelihood tree of the genus *Rhacophorus* based on partial 16S rRNA fragments. Numbers before slashes indicate ultrafast bootstrap support for Maximum Likelihood analyses (≥ 90 remain) and numbers after slashes indicate Bayesian posterior probabilities (≥ 0.90 remain).

IN 2.7 mm, UEW 2.7 mm); distance between anterior corners of eyes (DAE 6.5 mm) about 60.2% distance between posterior corners of eyes (DPE 10.8 mm); nostril oval, nearer the tip of snout; tympanum distinct, diameter (TYD 2.1 mm) less than eye diameter, tympanum separated from eye by distance (TYE 0.9 mm) 42.9% of tympanum diameter; pineal ocellus absent; supratympanic fold distinct, interrupted, extending from behind eye to beyond level of axilla, composed of large irregular glandular ridges; vomerine teeth arranged in two small oblique groups between choanae and widely separated from each other; tongue large, accounting for almost half of mouth, heart-shaped, dorsal surface smooth, notched and free posterior. Forelimbs slender (FLL 6.3 mm, HAL 16.2 mm); relative length of fingers: I < II < IV < III; tips of fingers

Table 2. Measurements (in mm) of the specimen of *Theioderma khoii* from Xichou County, Yunnan, China. For abbreviations see Materials and methods.

KIZRao201204		KIZRao201204	
SVL	29.4	TYD	2.1
HW	12.7	TYE	0.9
HL	12.4	FLL	6.3
SNL	4.9	HAL	16.2
ED	3.8	OPT	1.1
UEW	2.7	TFL	6.9
IN	2.7	FeL	15.7
IOD	3.4	TbL	15.8
DAE	6.5	FoL	13.3
DEP	10.8	FTL	7.0
EN	3.4	IMT	1.7

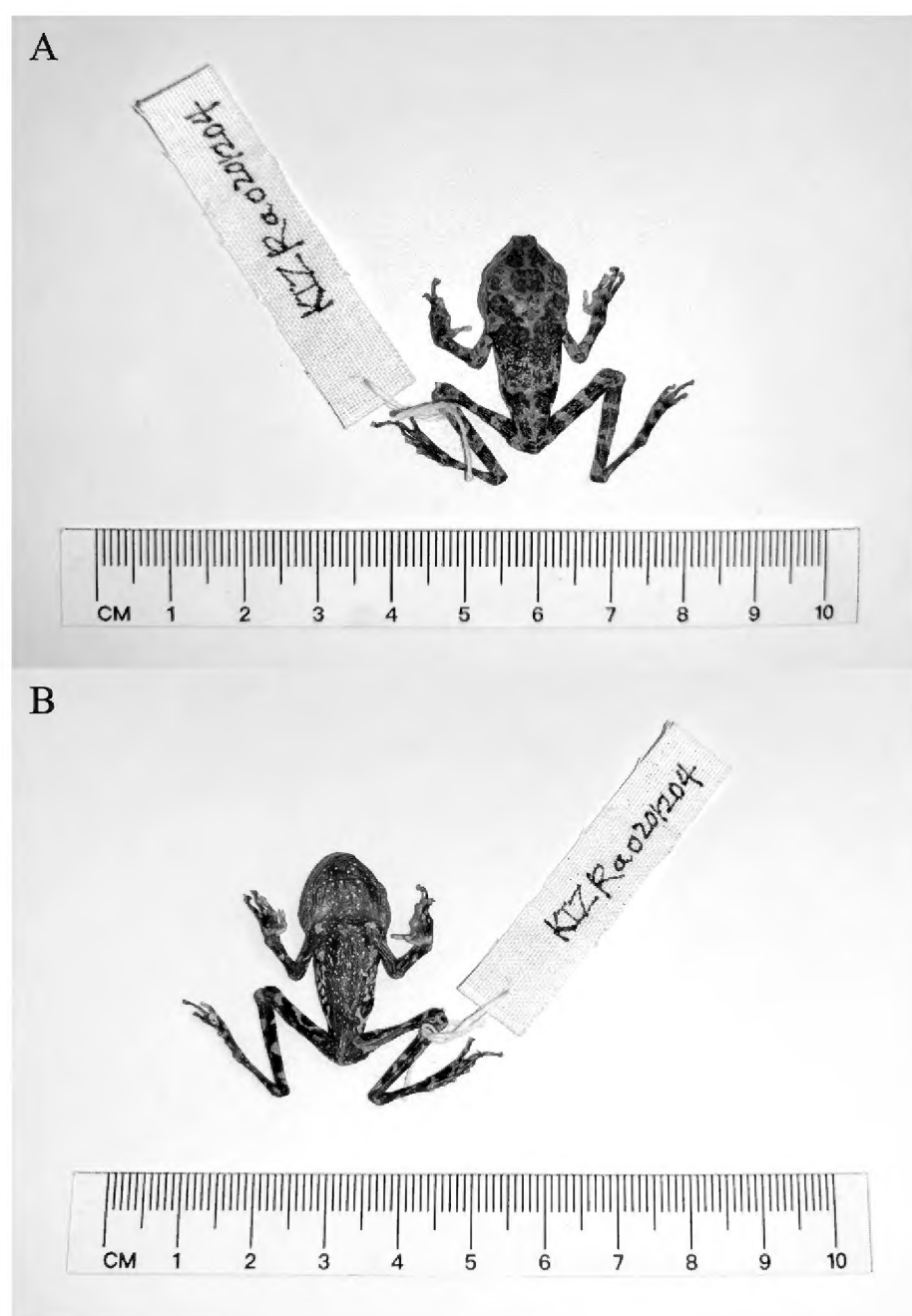


Figure 4. The specimen of *Theloderma khoii* from Xichou County, Yunnan, China, in preservative. **A.** Dorsal view; **B.** Ventral view.

dilated into large, rounded and flattened discs; subarticular tubercles large and distinct, with indistinct supernumerary tubercles; formula of subarticular tubercles: 1, 1, 2, 2; outer edge of fourth finger distinctly serrated. Hindlimbs slender, tibia longer than femur length and foot length (TbL 15.8 mm, FeL 15.7 mm, FoL 13.3 mm), tibiotarsal articulation reaching posterior edge of snout when hindlimbs pressed forward; relative length of toes $I < II < V < III < IV$; webbing formula $I(1/3) - (1)II(0) - (1/2)III(0) - (1)IV(1) - (1/3)V$; tips of toes dilated into rounded, flattened discs, smaller than discs on fingers; large subarticular tubercles roundish, formula of subarticular tubercles: 1, 1, 2, 3, 2; large inner metatarsal tubercle oval and raised (IMT 1.7 mm), outer metatarsal tubercle absent; outer edge of fifth toe with five distinct serrations.

Dorsal surface of head, body, and limbs very rough, with large irregular glandular ridges ordered symmetrically in middle vertebral region; a large, horn gland behind head roughly in X-shape; large conical tubercles on back of thighs near vent; lateral parts of body become granular, dense small tubercles below eye and 4–5 large tubercles behind tympanum on each side; throat and chest relatively smooth; belly and ventral surface and underside of thigh with thickened flat granules; axillary region and underside of tibia smooth.

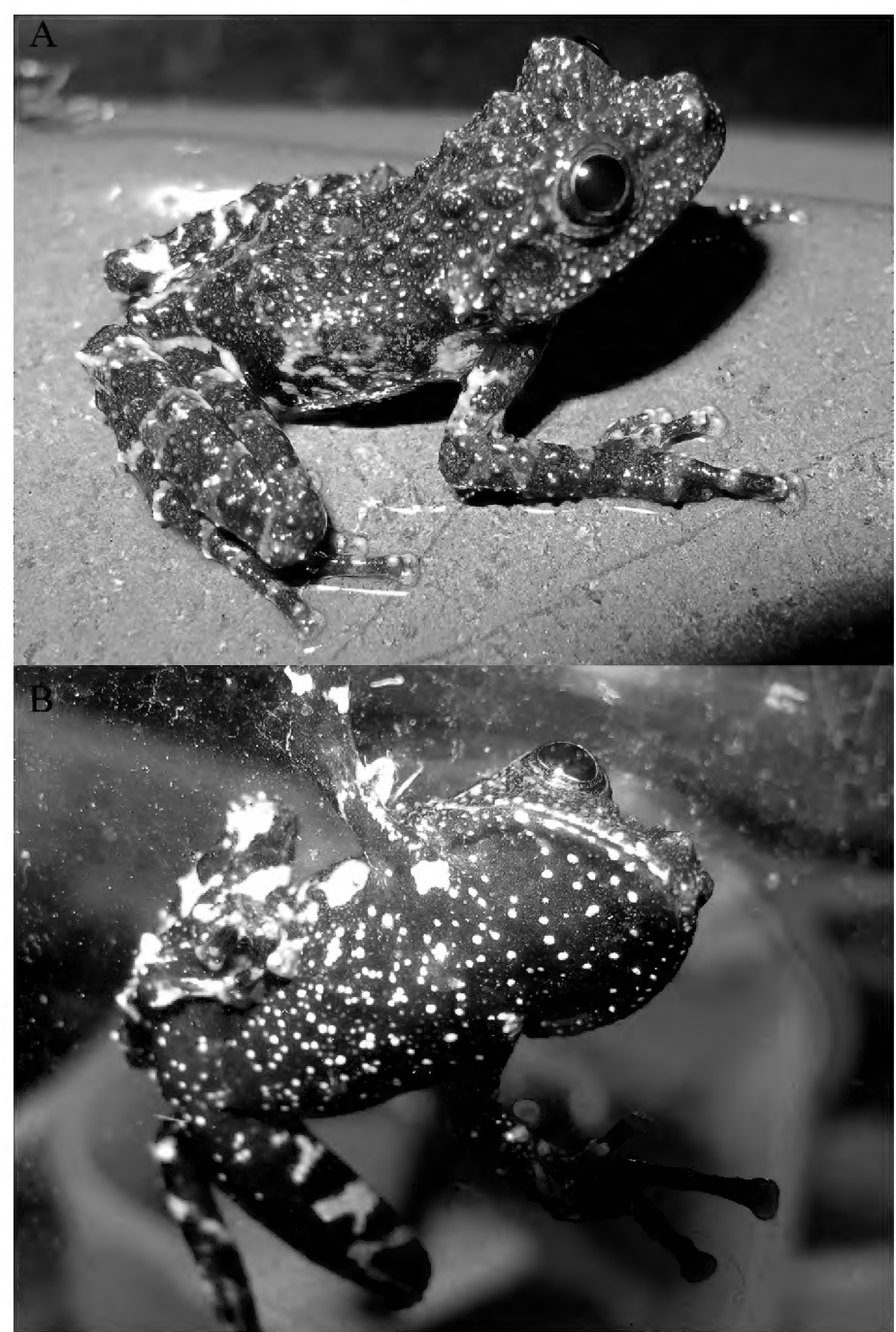


Figure 5. The specimen of *Theloderma khoii* from Xichou County, Yunnan, China, in life. **A.** Dorsolateral view; **B.** Ventral view.

Coloration in life. Dorsal surface of head and body green with irregular olive patches; tips of most tubercles red on olive patches and light green on other regions; dorsal surfaces of limbs green with dark olive bands, tips of tubercles red on bands and light green on other regions, finger and toe discs light green; lateral surface of head green, iris yellowish green with irregular black reticulation; tympanum dark olive; flanks light yellow with black patches and strips; ventral surface of head and body brownish black with many small white dots; a light yellow patch on anterior region of the base of forelimb on each side; ventral surface of limbs brownish black with some small white dots on ventral thigh and some light yellow patches on other regions of ventral limbs.

Distribution. This species is currently known only from the type locality in Ha Giang Province, northeastern Vietnam, and Xichou County, Wenshan Prefecture, southeastern Yunnan, China.

Rhacophorus orlovi Ziegler & Köhler, 2001

Figs 6, 7

Specimen examined. KIZ20210506, subadult female; KIZ20210507–KIZ20210508, two adult female; all collected on 18 May 2021 by Shuo Liu from Guiliang

A



B



Figure 6. The specimens of *Rhacophorus orlovi* from Hekou County, Yunnan, China, in preservative. **A.** Dorsal view; **B.** Ventral view.

Village, Laofanzhai Township, Hekou County, Honghe Prefecture, Yunnan Province, China (22°41'10"N, 103°49'45"E, 750 m elevation).

Description of the specimens from China. Measurements are presented in Table 3. Body size moderate (KRL 53.9–55.6 mm in adult females); head slightly longer than wide (KL/KB 1.01–1.02); snout slightly pointed, rounded in dorsal and lateral views; nostril closer to tip of snout

than to eye (NS/AN 0.59–0.83); canthus rostralis well developed, slightly rounded, constricted; loreal region concave, sloped towards lip; interorbital region slightly convex; interorbital distance longer than upper eyelid length and internarial distance; eye diameter shorter than snout; tympanum distinct, approximately half of eye diameter (TD/AD 0.48–0.52); vomerine teeth in oblique ridges; tongue cordiform, free behind.

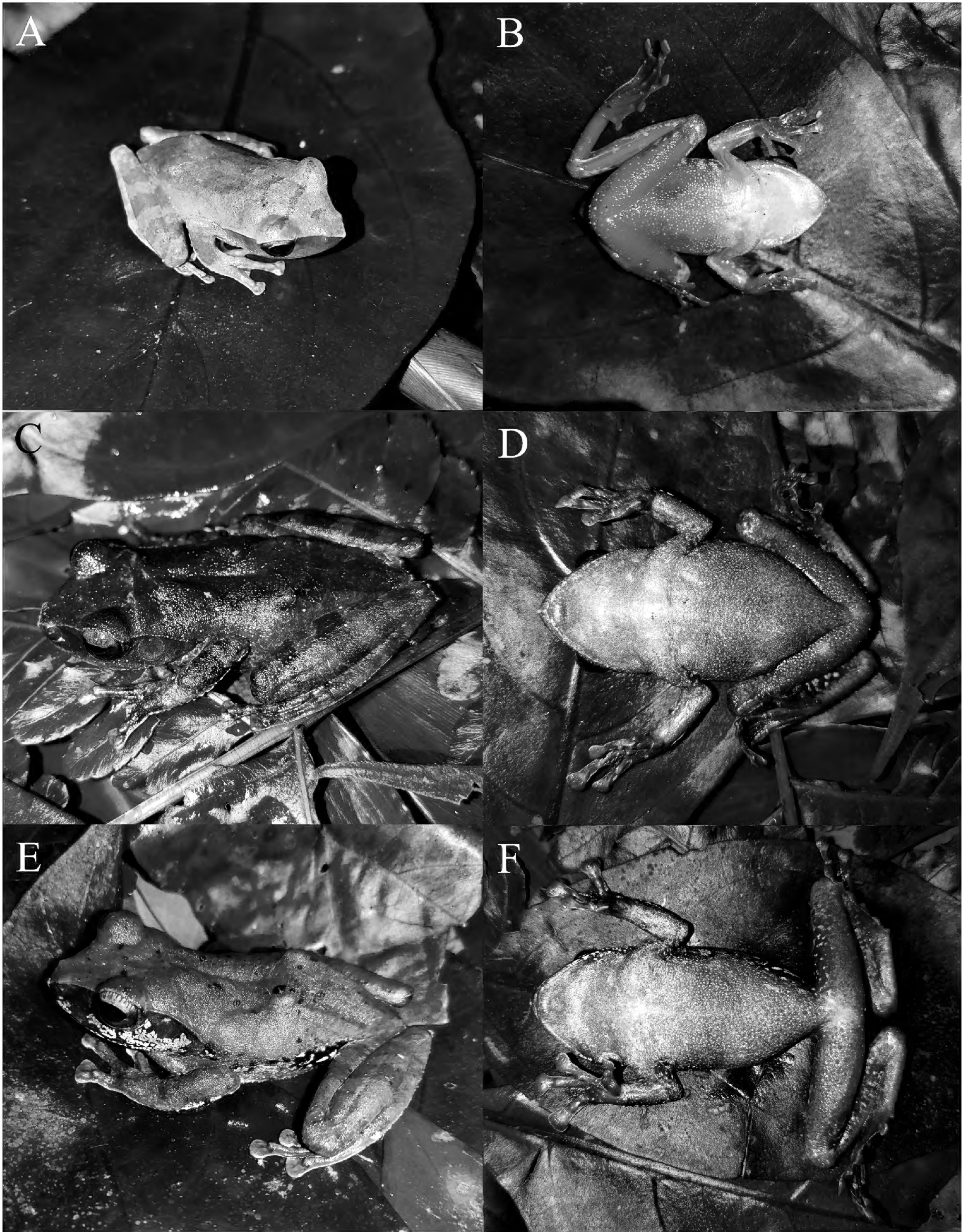


Figure 7. The specimens of *Rhacophorus orlovi* from Hekou County, Yunnan, China, in life. **A.** Dorsolateral view of the subadult female (KIZ20210506); **B.** Ventral view of the subadult female (KIZ20210506); **C.** Dorsolateral view of the adult female (KIZ20210507); **D.** Ventral view of the adult female (KIZ20210507); **E.** Dorsolateral view of the adult female (KIZ20210508); **F.** Ventral view of the adult female (KIZ20210508).

Forelimbs slender, relative lengths of fingers $I < II < IV < III$; subarticular tubercles large and distinct, with small and distinct supernumerary tubercles; formula

of subarticular tubercles: 1, 1, 2, 2; tips of fingers dilated into well developed, broad discs with circumferential groove; disc of third finger approximately equal

Table 3. Measurements (in mm) of the specimens of *Rhacophorus orlovi* from Hekou County, Yunnan, China. For abbreviations see Materials and methods.

	KIZ20210506	KIZ20210507	KIZ20210508
KRL	30.6	53.9	55.6
KB	12.1	19.9	20.9
KL	12.3	20.3	21.1
AN	3.0	5.6	6.0
NS	2.5	3.3	3.9
AD	4.0	6.5	7.3
TD	1.9	3.4	3.6
HaL	9.4	15.8	17.1
BL	53.1	87.1	90.7
FL	13.1	22.5	23.2
IMT	1.4	2.6	2.6

to tympanum diameter; webbing between first and second finger reaching subarticular tubercles; webbing between second and third finger nearly reaching base of disc of second finger and proximal subarticular tubercle of third finger; and webbing between third and fourth finger reaching distal subarticular tubercles. Hindlimbs slender, tibiotarsal articulation reaching between eye and snout tip when hindlimbs pressed forward; relative length of toes $I < II < III < V < IV$; subarticular tubercles distinct; formula of subarticular tubercles: 1, 1, 2, 3, 2; inner metatarsal tubercle oval, outer metatarsal tubercle absent; discs on toes smaller than those on fingers; webbing between first and second toe reaching base of disc of first toe and subarticular tubercle of second toe; webbing between second toe and third toe reaching base of disc of second toe and distal subarticular tubercle of third toe; webbing between third and fourth toe reaching base of disc of third toe and distal subarticular tubercle of fourth toe; and webbing between fourth and fifth toe reaching bases of discs.

Skin smooth dorsally and laterally with sparse tiny tubercles; supratympanic fold distinct, ending above insertion of arm; skin on throat smooth, on abdomen and inner thighs coarsely granular; weak tubercles and protuberances on outer edges of lower arms and tarsus; tarsal projections on heels indistinct; skin beneath anal opening and on posterior of thigh with few small whitish tubercles.

Coloration in life. Dorsum flesh color, reddish brown, or dark brown, with some black dots or indistinct stripes; dorsal limbs with distinct dark bands; loreal region dark brown with irregular light yellow patches or no patch; iris golden in upper third, bronze in remainder; flanks brown with indistinct reticulation or black and yellow spots; groin and lateral thighs and shanks brownish black with white spots; vent region greyish black; venter greyish in adult females and light yellow to orange in subadult female.

Distribution. This species is currently known from Vietnam and Laos, as well as Maguan County, Wenshan Prefecture, and Hekou County, Honghe Prefecture, Yunnan, China.

Discussion

Theloderma khoii was known previously only from northern Vietnam (Ninh et al. 2022). This is the first record of *T. khoii* from China and from outside Vietnam. The new locality in China is approximately 35 km away from the type locality in Vietnam (Fig. 4). Our work brings the total species number of the genus *Theloderma* to 11 in China and to eight in Yunnan.

The population density of *Theloderma khoii* in China may be very low, or this species is very infrequently encountered due to its cryptic habits. We collected a specimen of this species ten years ago, thereafter, we have conducted many field surveys in southeastern Yunnan, but we have never seen this species again. Since we have only one subadult specimen, we have not described it as a new species. Recently, this species was described and named from northern Vietnam, and we confirmed that the specimen we collected in southeastern Yunnan ten years ago is conspecific with this species. Therefore, we reported the first record of this species in China.

Hou et al. (2017) considered that *Theloderma bicolor* is distributed in Xichou County, Wenshan Prefecture, Yunnan, China, and provided a photograph (fig. 27 B) of *T. bicolor* from Xichou taken by Yi Fan. After verification, we confirmed that the individual in the photograph (fig. 27 B) in Hou et al. (2017) is the same one (KIZ-Rao201204) reported in this study. Therefore, we remove the record of *T. bicolor* from Xichou herein.

Little is known about the natural history of this species in China at present. More field surveys are needed to learn about and protect the populations of this species in China.

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References

- Du LY, Wang J, Liu S, Yu GH (2022) A new cryptic species in the *Theloderma rhododiscus* complex (Anura, Rhacophoridae) from China–Vietnam border regions. *ZooKeys* 1099: 123–138. <https://doi.org/10.3897/zookeys.1099.80390>
- Frost DR (2022) Amphibian Species of the World: an Online Reference. Version 6.1. Electronic Database. <https://amphibiansoftheworld.amnh.org/index.php> [Accessed on 20 May 2022]
- Gao ZF, Sun H (2016) Biological species list of Yunnan Province (2016 Edition). Yunnan Science and Technology Press, Kunming, 598 pp.
- Gao ZF, Sun H (2017) Species red list of Yunnan Province (2017 Edition). Yunnan Science and Technology Press, Kunming, 705 pp.
- Hou M, Yu GH, Chen HM, Liao CL, Zhang L, Chen J, Li PP, Orlov NL (2017) The taxonomic status and distribution range of six *Theloderma* species (Anura: Rhacophoridae) with a new record in China. *Russian Journal of Herpetology* 24(2): 99–127. <https://doi.org/10.30906/1026-2296-2019-24-2-99-127>
- Jiang DC, Jiang K, Ren JL, Wu J, Li JT (2019) Resurrection of the genus *Leptomantis*, with description of a new genus to the family Rhacophoridae (Amphibia: Anura). *Asian Herpetological Research* 10: 1–12.
- Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software, version 7: improvements in performance and usability. *Molecular Biology and Evolution* 30: 772–780. <https://doi.org/10.1093/molbev/mst010>
- Kropachev II, Evsyunin AA, Orlov NL, Nguyen TT (2022) A New Species of *Rhacophorus* Genus (Anura: Rhacophoridae: Rhacophorinae) from Lang Son Province, Northern Vietnam. *Russian Journal of Herpetology* 29(1): 35–46. <https://doi.org/10.30906/1026-2296-2022-29-1-35-46>
- Li JT, Li Y, Klaus S, Rao DQ, Hillis DM, Zhang YP (2013) Diversification of rhacophorid frogs provides evidence for accelerated faunal exchange between India and Eurasia during the Oligocene. *Proceedings of the National Academy of Sciences of the United States of America* 110(9): 3441–3446. <https://doi.org/10.1073/pnas.1300881110>
- Liu S, Hou M, Mo MZ, Rao DQ (2021) A new species of *Micryletta* Dubois, 1987 (Anura, Microhylidae) from Yunnan Province, China. *Herpetozoa* 34: 131–140. <https://doi.org/10.3897/herpetozoa.32.e69755>
- Ninh HT, Nguyen TT, Nguyen HQ, Hoang NV, Siliyavong S, Nguyen TV, Le DT, Le QK, Ziegler T (2022) A new species of mossy frog (Anura: Rhacophoridae) from Northeastern Vietnam. *European Journal of Taxonomy* 794: 72–90. <https://doi.org/10.5852/ejt.2022.794.1655>
- Palumbi SR, Martin A, Romano S, McMillan W, Stice L, Grabowski G (1991) *The Simple Fool's Guide to PCR*. University of Hawaii Press, Honolulu, 94 pp.
- Poyarkov NA, Kropachev II, Gogoleva SI, Orlov NL (2018) A new species of the genus *Theloderma* Tschudi, 1838 (Amphibia: Anura: Rhacophoridae) from Tay Nguyen Plateau, central Vietnam. *Zoological Research* 39: 158–184. <https://doi.org/10.24272/j.issn.2095-8137.2018.018>
- Poyarkov NA, Nguyen TV, Popov ES, Geissler P, Pawangkhanant P, Neang T, Suwannapoom C, Orlov NL (2021) Recent progress in taxonomic studies, biogeographic analysis, and revised checklist of amphibians of Indochina. *Russian Journal of Herpetology* 28(3A): 1–110. <https://doi.org/10.30906/1026-2296-2021-28-3A-1-110>
- Sambrook J, Fritsch EF, Maniatis T (1989) *Molecular cloning: A laboratory manual*. 2nd Edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, 1659 pp.
- Ziegler T, Köhler J (2001) *Rhacophorus orlovi* sp. n., ein neuer Ruderfrosch aus Vietnam (Amphibia: Anura: Rhacophoridae). *Sauria* 23: 37–46.